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Research Highlights

Innovative 21st-century roadmaps need to be devised for biomedical research.

Advanced human biology-based models and tools hold the key to progress.

Human disease pathways mapped at multiple biological scales are an important concept.

Funding should focus on acquiring critical human data instead of on animal models.

Towards a 21st-century roadmap for biomedical research and drug discovery: consensus report and recommendations

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Teaser: To discover and develop new therapies, we need 21st-century roadmaps for biomedical research based on multiscale human disease pathways, and supported by policy and funding strategies that prioritise human relevance.

Author biographies

Gillian Langley
Gillian Langley is currently a scientific consultant to Humane Society International. Her academic career focussed on neurochemistry at Cambridge University, while at Nottingham University, she specialised in studying signalling pathways in human neural cells *in vitro*. Subsequently, she led science programs at the Dr Hadwen Trust for Humane Research, a medical charity developing human-specific disease models and

research techniques. Gill has been a member of the British Government's advisory committee on animal experiments, and was an adviser on non-animal safety tests during the development of the European chemicals legislation (REACH) and a member of European Commission expert subgroups on non-animal testing.

Alysson Muotri

Alysson Muotri is a professor at the University of California San Diego and director of the UCSD Stem Cell Program. His research focuses on human brain development and evolution, and utilises a range of advanced models and molecular tools to study neurological diseases, such as autism spectrum disorders. Using human induced pluripotent stem cells, Alysson's team has developed several techniques to culture human neurons and glia for basic research and drug screening. He is a recipient of numerous awards, including the NIH Director's New Innovator Award.

Martin Hofmann-Apitius

Martin Hofmann-Apitius is head of Department of Bioinformatics at the Fraunhofer Institute for Algorithms and Scientific Computing, and professor of applied life science informatics at Bonn-Aachen International Center for Information Technology. Martin's current research focuses on automated methods for extracting relevant information from unstructured information sources, such as journal publications, patents, and web-based sources, as well as knowledge-based, mechanistic modelling of neurodegenerative diseases (including the first comprehensive, computable model of Alzheimer's disease), and mining in real-world data (social networks, patient fora, and electronic patient records). He is the initiator and academic co-ordinator of the Innovative Medicines Initiative project 'AETIONOMY'.

Decades of costly failures in translating drug candidates from preclinical disease models to human therapeutic use warrant reconsideration of the priority placed on animal models in biomedical research. Following an international workshop attended by experts from academia, government institutions, research funding bodies, and the corporate and nongovernmental organisation (NGO) sectors, in this consensus report, we analyse, as case studies, five disease areas with major unmet needs for new treatments. In view of the scientifically driven transition towards a human pathway-based paradigm in toxicology, a similar paradigm shift appears to be justified in biomedical research. There is a pressing need for an approach that strategically implements advanced, human biology-based models and tools to understand disease pathways at multiple biological scales. We present recommendations to help achieve this.

Introduction

To date, the discovery and development of new drugs have relied heavily on the use of preclinical animal models. However, it is widely recognised that this reliance on animal models, which recapitulate only limited aspects of human disease, is holding back progress in many areas [1,2]. The average cost of research and development for a successful drug is estimated to be US$2.6 billion [3] and the number of new drugs approved per billion US dollars spent has halved roughly every 9 years since 1950, decreasing around 80-fold in inflation-adjusted terms [4]. More than 90% of compounds entering clinical trials fail to gain regulatory approval, mainly as a result of insufficient efficacy and/or unacceptable toxicity, because of the limited predictive value of preclinical studies [5].

The failure of animal studies to predict drug efficacy and toxicity in humans has several causes, including experimental design flaws and bias, but species variations are the most significant [6]. There is a growing recognition that, to increase the success rate, a stronger focus on human-relevant data is needed [7,8]. Animal research can provide useful *in vivo* data about selected pathologies already identified as significant, usually from human-specific studies. However, increasingly, this research could be conducted using innovative human- and disease-specific models and tools, thereby also eliminating the unsolvable problems of species variations.

To address the animal-model challenges of poor predictivity and cost-efficiency limitations, as well as incomplete knowledge of human biological pathways, toxicologists are beginning to move away from observing adverse effects in whole-animal models. Instead, they are looking towards measuring *in vitro* early endpoints that are predictive of adverse effects, using human cellular and molecular assays. This pathway-based approach is embedded in an 'adverse outcome pathway' (AOP) framework, with a focus on the species of ultimate regulatory interest, that is humans rather than rodents, for human health risk assessment [8,9]. This framework allows the modelling of normal cellular signalling pathways that are perturbed by chemical exposure, and captures the consequential changes occurring at multiple biological levels in an individual, which eventually lead to adverse effects. An AOP is a conceptual construct representing existing knowledge to describe a sequence of causally linked events. This starts at the molecular level and progresses through different levels of biological organisation to an adverse health outcome in an individual (or population) [9].

Understanding AOPs in humans is enabling the emergence of a new predictive toxicology paradigm and has been adopted as part of the strategic research plan of the US Environmental Protection Agency (EPA) [10], and globally by the Organisation for Economic Cooperation and Development (OECD) [11]. The OECD AOP Development Program coordinates the efforts of its 34 member countries, offering a forum for international cooperation, common guidance (e.g., User Handbook; http://aopkb.org/common/AOP_Handbook.pdf) and

knowledge management tools (e.g., the AOP Knowledge Base; http://aopkb.org).The OECD activity was initiated by toxicologists with the goal of providing improved tools for regulatory risk assessment, but the basic biology under investigation is the same as in biomedical research. Thus, both communities could benefit from the consolidation of expertise into common knowledge bases using a shared lexicon.

A workshop entitled 'BioMed21: A Human Pathways Approach to Disease Research', convened in December 2015 in Brussels, brought together leading health scientists, officials representing European institutions, national regulatory and research agencies, science-funding organisations, and other stakeholders, to discuss these issues. As case studies, research was examined into Alzheimer's disease (AD), autism spectrum disorders (ASD), cholestatic liver diseases (CLDs), respiratory diseases, and autoimmune diseases. Also considered were research funding frameworks and regulatory structures in the European Union (EU) and the USA. The workshop goal was to identify actionable consensus recommendations (Box 1) as a first step towards a comprehensive roadmap for human biology-based health research, as well as funding and policy frameworks.

Adverse outcome pathways

Some will argue that next-generation, human-specific techniques should complement rather than replace animal models in biomedical research and drug discovery [12]. However, a paradigm shift away from animal-model reliance, as is happening in toxicology, is essential to overcome roadblocks in knowledge of human diseases and the discovery of effective therapies [13,14]. In 2016, the EU launched EU-ToxRisk, a €30 million flagship project to drive forward mechanism-based toxicity testing and risk assessment for the 21st century (www.eu-toxrisk.eu/). The project consortium includes many of Europe's leading toxicologists and experts in related fields; the project launch release stated: 'These new concepts involve cutting-edge human-relevant *in vitro* non-animal methods and *in silico* computational technologies to translate molecular mechanistic understanding of toxicity into safety testing strategies. The ultimate goal is to deliver reliable, animal-free hazard and risk assessment of chemicals'.

Adapting the AOP concept, a critical part of the current transition underway in toxicology, would have great value for health research, drug discovery, and drug efficacy and safety testing. Mapping the perturbation of normal human biological pathways would, in many cases, be applicable both to efficacy and toxicity assessment, depending on whether the effect is desired. Thus, the disease pathways we envisage would link molecular initiating events through key events in cells, organs, and systems to disease outcomes in individuals (Figure 1). The AOP approach would help assign value to potential drug targets at an early stage (with obvious cost and time advantages), according to their roles in multiscale disease pathways, rather than merely considering an isolated disease mechanism or a solely molecular or cellular pathway [14].

Alzheimer's disease

AD is the most common form of dementia and is increasing in prevalence as human populations age. It is characterised by cognitive decline with distinctive brain pathologies, including regional loss of neurons with accumulation of amyloid beta (Aβ) plaques and neurofibrillary tangles of hyperphosphorylated tau. These are accompanied by chronic inflammation and extensive oxidative damage. Dozens of strains of transgenic mice have been the dominant models in AD research for 15 years and have contributed to understanding some of the disease pathways underlying the condition. However, between 1998 and 2011, 100 compounds failed in clinical trials despite encouraging preclinical results [15] and none of 300 interventions tested in a transgenic mouse model, Tg2576, has gone on to clinical trials [16]. There have been no new therapies for AD for 10 years and the five licensed drugs stabilise symptoms temporarily in only about half of patients and do not slow progression of the disease.

Reasons for the failure of potential new drugs for AD in clinical trials are multifactorial, but a major issue is the overdependency on inadequate animal models. Transgenic mouse models and transformed mammalian cell lines (widely used in drug screening and optimisation) substantially overexpress mutant proteins, and the simplistic cell models may not accurately represent native human neurons and their interactions with other brain cell types. These limitations contribute to drug development failures [17]. Transgenic mice only partially recapitulate the pathophysiology and aetiology of human AD, have a limited behavioural or cognitive repertoire, and have poor predictive validity for human AD [18].

It is essential to move away from inadequate animal models and simplistic cell systems and instead develop innovative human-specific approaches. These will allow a better understanding of normal human biology, more closely mimic disease pathology, and better recapitulate underlying disease pathways [19]. Significant progress is being made with human induced pluripotent stem cells (hiPSCs) generated from donated somatic cells and differentiated into disease-specific and patient-specific neural cells. In one such model, neural cells derived from patients' iPSCs expressed forebrain and neocortical markers as well as amyloid precursor protein (APP), β-secretase, and γ-secretase (involved in Aβ production), and secreted Aβ. Aβ production was reduced by secretase inhibitors and a nonsteroidal anti-inflammatory drug, suggesting that the model could be developed as a drug screen [20].

Patient-derived iPSCs differentiated into neural cells have been used to investigate AD-associated gene regulatory networks [21]. Phosphorylated tau was expressed and transcriptome analysis revealed significant gene expression changes associated with AD. This system could be used to study the underlying molecular basis of sporadic AD and for drug screening and toxicology. A 3D human stem cell model of AD has also recapitulated key pathological features, including Aβ plaques and neurofibrillary tangles [22]. hiPSC-derived

neural cells are being developed as a microfluidic human brain model *in vitro*, which will likely have applications in research into AD, ASD, and other neurological disorders [23]. Microfluidic platforms with incorporated optogenetic neural cell stimulation enable high-resolution, light-controlled stimulation of individual neural cells to permit millisecond-by-millisecond studies of activity *in vitro* [24]. Collectively, this research reveals the potential of human neural models derived from hiPSCs to improve opportunities to study AD initiation and pathogenesis. This will clarify the roles of different cell types and identify potent and safe compounds preclinically. This would help to avoid failure in later-stage and costly *in vivo* animal or clinical studies.

Systems biology tools and computational modelling have important roles in integrating and interpreting multilayered, human-specific research data and in helping to unravel complex, nonlinear biological processes. The AETIONOMY project combined computational disease models with curated data and additional functionalities, such as literature mining, to support rational approaches for identifying disease mechanisms in neurodegenerative disorders [25]. A model for AD is based on biological expression language (BEL), which integrates molecular data, clinical information, neuroimaging data, and cognitive testing readouts. Initially, the project generated two mechanistic hypotheses [26] and, subsequently, has delivered more than 120 candidate mechanisms potentially involved in AD aetiology.

State-of-the-art clinical brain imaging can now generate high-resolution human structural and functional information, offering progress in finding novel, non-invasive imaging biomarkers for both the clinic and for drug discovery. Neuroimaging enables detailed studies of AD in humans *in vivo*, arguably the 'gold standard' model for any disease [20]. To maximise the value of human neuroimaging studies, the gap between clinical imaging and molecular biology needs to be bridged. A new computational method for AD can link neuroimaging biomarkers to their underlying molecular pathways. The approach integrates brain region-specific molecular interactions, drug target information, and biomarker expression. This combined analysis approach can help identify relevant drug targets and reduce the risk of failure in clinical trials, because it uses human data instead of animal data that poorly reflect human neurodegenerative conditions [19].

Taken together, all these approaches demonstrate the feasibility of mapping human disease pathways at multiple scales. Integrating human *in vitro*, *in vivo*, and *in silico* data will enrich our understanding of human disorders and aid the discovery of new drug targets (Figure 2). Representing the current state of knowledge in computational models using a human pathways approach will enable the identification of knowledge gaps and the integration of new data into existing knowledge. As a result, we will build a more complete picture of complex disease pathophysiology.

Autism spectrum disorders

ASD are lifelong developmental disabilities characterised by persistent deficits in social communication and interaction, and restricted, repetitive, and stereotyped patterns of behaviour. Different aetiologies can cause a similar behavioural outcome; consequently, many diseases with autistic features are grouped within ASD. The prevalence has increased dramatically over recent years. There are no specific therapies and most people with autism cannot live independently, underlining the need for better treatment options. The intrinsically human nature of ASD, its genetic heterogeneity, and the spectrum of clinical symptoms, together with a historical lack of living human brain cells for research, have, until recently, prevented progress in understanding disease pathways and in developing treatment approaches. The inherent differences between mouse and human genetic backgrounds and brain circuitry limit the value of rodent models of ASD [28[DE1]]. Drug discovery success for neurological disorders generally has been poor, mainly because of the lack of predictive validity of animal models [12].

Human *in vitro* models using reprogrammed patient somatic cells are an attractive option, because they capture a patient's genome in relevant cell types and can recapitulate early stages of brain development. Given the unique nature of human cognition and behaviour, an *in vitro* human neurodevelopmental model could reveal biochemical and cellular features of conditions, such as ASD, that are difficult to reproduce in other models. Patient-specific iPSCs from individuals with confirmed diagnoses represent a powerful new approach, capturing both the primary genetic change and the genetic background [12]. Human stem cell biology, tissue engineering, bioinformatics, and machine learning have been combined to create an *in vitro* human cellular model for developmental neurotoxicity screening [29]. The model comprises human embryonic stem cell-derived 3D neural constructs with vascular networks and microglia in a chemically defined synthetic hydrogel scaffold, which improves reproducibility. In a blinded trial, nine out of ten chemicals were correctly classified and the model could be useful in drug as well as chemical toxicity assessment.

Marchetto and colleagues used hiPSC-derived neurons and glial cells as a genetic model of ASD, to study Rett syndrome (RTT) [30]. RTT is a severe form of ASD caused by a genetic alteration in the gene encoding methyl-CpG-binding protein-2 (*MeCP2*). It presents with neurodevelopmental and speech delays that result in an autistic phenotype [30]. iPSC-derived neurons from patients with RTT showed reduced glutamatergic excitatory synapses, decreased frequency of spontaneous postsynaptic currents (as measured by whole-cell patch-clamping), and altered neuronal network connectivity. RTT neurons also had smaller neuronal somas and lower spine densities, similar to those seen in RTT postmortem brain tissues. Treating these RTT neurons with insulin growth factor 1 *in vitro* increased the number of glutamatergic synapses, indicating that

such an approach could correct the RTT neuronal phenotype, as well as providing clues about dose and timing parameters. Thus. although still at a relatively early stage, hiPSC models have demonstrated the ability to recapitulate relevant neuronal defects occurring in ASD and show potential as drug-screening platforms [31].

hiPSC-derived RTT neuronal cultures and high-throughput robotics are being used with the aim of screening 55 000 small-molecule drug candidates that might rescue the RTT synaptic defects, by the end of 2016 (A. Muotri *et al*., unpublished results, 2016). Validation of these findings can be carried out with hiPSC-derived 3D neurospheres using multielectrode array assays, as in a recent study of a rare neurodevelopmental disorder called the MECP2 duplication syndrome [32]. A potential drug candidate not previously considered as a therapeutic candidate for neurological disorders appeared to rescue the aberrant morphology and ameliorate the functional phenotype [32]. These data indicate that hiPSCs can recapitulate some aspects of a neurodevelopmental disorder caused by genomic duplication, and can also be used in *in vitro* assays to screen potential drugs. hiPSC models allow manipulation of phenotype changes with candidate drugs, offering rapid, disease pathway-based, and species-specific drug screening platforms. Using mouse models, the research would have taken much longer and the results would not necessarily predict the human response.

For research into ASD and other diseases, innovative strategies are needed to collect biological material and clinical information, including biomarkers, from large patient cohorts. This will improve the validity of new models and increase the statistical power to distinguish information from noise caused by the variability introduced by reprogramming and differentiation methods. The EU-AIMS project of the Innovative Medicines Initiative (IMI) in Europe is a private/public initiative involving patient organisations in Europe and the USA, aiming to improve drug discovery for autism. Although much of this research is still focussed on mouse models, an important initiative is a European biobank. This has been set up to aid the generation of ASD-specific hiPSCs to produce differentiated, characterised neurons for autism research. Research into neurological disorders is already benefiting from hiPSC-disease modelling because of its capability for generating disease-relevant cell types *in vitro* from the central nervous system, previously only available from postmortem samples [33]. Data from other approaches, such as human brain imaging, population genetics, systems biology, and computational modelling, should be integrated to generate novel hypotheses that can be tested using stem cell-derived human brain cells.

Cholestatic liver diseases

CLDs involve perturbed bile acid homeostasis and impaired liver function resulting from the blockage of bile flow. Although genetic factors and adverse drug effects are the main causes of CLDs, the so-called

'diseases of civilisation', such as obesity and diabetes, are also implicated [34]. CLDs are often chronic and lead to overall liver damage. Progression to cirrhosis means that many patients with CLDs require liver transplantation. Given that few therapeutic options currently exist (mostly symptomatic relief), there is a pressing need to develop new treatment approaches.

Despite studies in many animal models and substantial clinical data, there have been limited advances in developing treatments for CLDs. Animal models have provided mechanistic insights into CLDs, but direct extrapolation of animal data to human physiology has been challenging. This is because of significant species differences in gut and liver physiology and in pathogenesis. *In vivo* animal models differ from humans in bile acid composition, transporter activities, immune and inflammatory responses, cytochrome P450 enzymes, gut microbiota, and mechanisms of parenchymal injury [34]. By contrast, current *in vitro* models are generally 2D monocultures, unrepresentative of the 3D architecture of liver tissue, and are often derived from rodent primary cells or transfected cells. *In vitro* CLD models should ideally reflect the complexity of human *in vivo* liver function.

Over the past few years, there have been rapid developments in cell culture and analytical techniques that enable CLDs to be studied *in vitro.* These include using human-derived cells, such as primary hepatocytes, genetically modified human cell lines, and hiPSC-derived liver models. For example, differentiated human HepaRG cells (a human hepatoma cell line) are increasingly being used in drug uptake, metabolism, and elimination studies [35]. HepaRG cells have metabolic activity and functional main hepatobiliary transporters [36]: in a study involving drug-induced cholestasis, they were considered suitable for investigating pathophysiological mechanisms of CLDs [37].

A 3D environment allows the improved expression of metabolising enzymes and transporters *in vitro* [38]. In 3D cultures, HepaRG cells develop a network of bile canaliculi and functional bile acid transporters, offering an important tool for studying CLDs. In the EU-NOTOX project of the Safety Evaluation Ultimately Replacing Animal Testing (SEURAT) initiative, HepaRG cells were used to investigate cholestasis in acute and chronic situations, focussing particularly on the effect of bile acid load and the mechanisms of drug-induced cholestasis. Chlorpromazine, known to induce CLD, disrupted the bile canaliculi network in 3D HepaRG spheroids, which confirmed their value in studying drug effects [39]. Exposure of HepaRG cells to bile acids *in vitro* caused cell death by necrosis, as mainly occurs in human CLDs. This contrasts with the apoptosis seen in rodent hepatic cells, further supporting these human-specific models [40]. 3D multicellular systems could help bridge the gap between conventional 2D models and *in vivo* clinical studies in humans.

Other human-specific *in vitro* models include primary human hepatocytes, precision-cut liver slices, and liver cells derived from hiPSCs, supported by technologies including perfusion bioreactors and microfluidic

platforms. These enable high-throughput screening, high-content imaging, and computer modelling, as well as the application of the 'omics *in vitro*. A human liver microfluidic device with variable perfusion rates has been developed that is compatible both with human primary hepatocytes and with patient-specific, iPSC-derived hepatocyte-like cells [41]. 3D organoids, with hepatocytes alone or in co-cultures, maintained stable hepatic function, such as albumin secretion and metabolic activity, for up to 28 days.

In liver research, as in other fields, there are conceptual and data gaps between *in vitro* findings and clinical knowledge and information that need to be bridged so that the different research communities can communicate and benefit from each others' work. The bridge could be via the defining of 'disease AOPs' that connect molecular- and cell-level data, through key events, to the clinical picture (Figure 3). Human data are the benchmark, and efforts are needed to expand clinical studies as well as histological analyses of *ex vivo* tissue samples (e.g., blood, serum, and urine) and postmortem tissues from patients with disease. Both liver disease research and liver toxicology can benefit from a synergistic approach based on the AOP framework. This provides a powerful tool that will assist in integrating data from various sources, aiding mechanistic understanding, identifying human biomarkers, locating uncertainties, and helping direct future research.

An AOP for drug-induced cholestasis was published in 2013. It spans events at molecular, cellular, organ, and individual levels, with bile salt export pump inhibition as the molecular initiating event (MIE) and cholestasis as the adverse outcome [42]. Key events include the accumulation of bile, the induction of oxidative stress and inflammation, and the activation of three nuclear receptors. Together with other intermediate steps, this MIE and subsequent key events drive both an adverse cellular response, which underlies directly caused cholestatic injury, and an adaptive cellular response to the primary cholestatic insults. Direct as well as secondary inducing and inhibiting effects of oxidative stress and/or inflammation are included. The AOP is highly relevant both to toxicology and liver disease research and illustrates the potential for the AOP concept to assist in understanding pathophysiology and identifying druggable targets.

Integrating data from the 'omics technologies and innovative human cell models, combined with computational modelling, will enhance understanding of CLD pathways and improve predictive modelling. A pathways-based systems approach is important for bringing together these emerging data and to develop novel therapy options. Additionally, better collaboration between healthcare professionals and scientists in different disciplines from academia and industry will also be fruitful.

Respiratory diseases: asthma and cystic fibrosis

Asthma is a common, complex, and heterogeneous condition, affecting an estimated 20% of children and 10–15% of adults. It is essentially a chronic inflammatory disease of the airways and, although most patients with asthma respond well to conventional anti-inflammatory agents, these are not a cure and the disease

returns when drugs are withdrawn. Five percent of patients with asthma do not respond to inhaled or even oral corticosteroids.

Few new drugs have reached the clinic over the past 50 years despite considerable research funding and effort. The reliance on animal models has signally failed to provide novel therapies that translate into humans [43,44]. A comprehensive understanding of asthma pathophysiology is still lacking and there is an excessive focus on asthma as an allergic inflammatory condition. both attributable, in large part, to a reliance on animal models. Many drugs that appear promising in preclinical animal studies fail in humans because of the lack of safety and/or efficacy, given that asthma is unique to humans. The common mouse models do not recapitulate the complexity of asthma and, thus, a new approach to drug discovery and development is necessary [45]. There are striking differences between animal species and strains compared with human asthma, in terms of genetic basis, anatomy, physiology, underlying mechanisms, pathological response, and responsiveness to drugs [46]. Mouse models offer an integrated pathophysiological system for studying airways inflammation and hyper-responsiveness, but these characteristics are notably different from those observed in patients with asthma [47].

While improvements in animal models are being made, these 'cannot bridge the chasm between the models and the myriad complexities of the human disorder and multiple asthma endophenotypes' [46]. A greater focus is needed on human-specific airways models *ex vivo* and *in vitro*, as well as the use of other clinical samples, to improve translational success. There is now growing recognition that not all patients with asthma are allergic and that asthma is a syndrome. This underlines the need for better research to understand the underlying mechanisms as well as the pathophysiology in different subsets of patients.

The Unbiased Biomarkers for the Prediction of Respiratory Disease Outcomes (U-BIOPRED) program, funded by the IMI, is taking a systems approach to researching severe asthma with the aim of mapping multiple 'omics data (e.g., metabolomics, proteomics, and genomics) onto tissue samples from patients and their clinical and physiological data. New bioinformatics and mathematical approaches are being applied to generate novel clinical asthma classifications and to enable novel disease-associated pathways to be investigated. As part of the U-BIOPRED project, patients with severe asthma from across Europe have been recruited and a range of their tissue samples, including plasma, urine, sputum, and biopsies, has been biobanked for the acquisition of 'omics data. Biomarker signatures, derived from a combination of clinical and high-dimensional biomarker data, are collected within a single technical platform. These data are being mapped to data from preclinical human cellular and tissue models, as well as mouse and guinea pig models. Combined clinical and biomarker data are also collected using multiple technical platforms. In a pioneering approach, machine learning is applied to create topological networks. This reveals statistically significant

patterns, including clustering of sputum lipidomics and proteomics data among patients, yielding several clear subgroups of asthmatics. A key finding was that eosinophil-predominant asthma was driven by at least two distinct clusters of genes. These clusters differed from those characteristic of neutrophilic and other types of asthma [48]. This could lead to the molecular phenotyping of patients and the identification of disease-relevant mechanisms, rather than the current clinical signs and symptoms approach, which has not resulted in effective treatment. A similar approach using topological data analysis has demonstrated clear subsets of patients with severe eosinophilic and neutrophilic asthma [49]. These data can be compared with other databases and signatures to assess different disease models and treatment effects. For example, although the chronic house dust mite mouse model uses a clinically relevant allergen and is reproducible, it is not predictive of steroid responsiveness in patients with severe asthma. Examining the enrichment of specific signatures associated with distinct asthma subphenotypes could indicate which, if any, this model mimics. These disease signatures can also be examined in some of the new 3D organoid culture systems being developed [50] and in combination with other structural and immune cells, particularly from patients.

Functional genomics helps clarify links between genotype and phenotype on a genome-wide scale. It also aids the study of molecular mechanisms and pathways of disease, including gene–environment interactions, and it offers a tool to generate novel hypotheses. Functional genomics studies in asthma have used endobronchial biopsies, epithelial brushings, bronchoalveolar lavage, and *ex vivo* cultured cells from patients with asthma and healthy controls. The results have led to the identification of potential new asthma subphenotypes, biomarkers, and treatment strategies [51,52]. The value of functional genomics would be improved if better bioinformatics programs were available to manage and interpret large-scale data sets. Promising future approaches include the study of more homogeneous, sorted cell populations or single human cells obtained by techniques such as laser capture microdissection. Together with functional genomics methods, these will capture benchmark patterns from multiple analyses of these cells.

Many asthma researchers see clear benefits to increasing the use of human tissue in research, including a greater knowledge of pathophysiology and quicker development of effective new therapies [44]. Access to normal and diseased human tissue is seen as a major barrier with significant logistical hurdles to overcome. Worldwide, human lung tissue is wasted; better cooperation is needed between pathologists, transplant surgeons, and researchers to increase its availability for research. This is an important issue for all disease areas. Progress has been made, for example, in heart research, where multiscale functional physiological data have been obtained from explanted human hearts *in vitro* using an array of imaging modalities. This has already indicated significant differences between animal models and human heart disease and provides a quantitative foundation for multiscale physiological models of the human heart [53]. Half of asthma

researchers surveyed agreed that journals need to be willing to publish human tissue research without accompanying animal model data [44]. This would increase the evidence base supporting the use of human tissue models and provide confidence to encourage their wider uptake.

A group at the University of Aston, Birmingham, UK, has developed a multicellular *in vitro* model of human airways for research into cystic fibrosis (CF). The healthy model incorporates human pulmonary fibroblasts in human type IV placental collagen on a porous membrane above culture medium, the fibroblasts overlaid with ciliated airways epithelial cells with an air–liquid interface [54]. The epithelium is stratified and differentiated and tight junctions apically provide a resistant barrier, permitting analysis of whether responses occur apically or basally. There are functional cilia and mucus secretion, and the model replicates the mucociliary 'escalator' that functions *in vivo*.

When populated with a CF airways epithelial cell line, the model showed similarities to the human disease, including a hyperinflammatory response with increased production of proinflammatory mediators compared with the healthy lung model (L.J. Marshall *et al*., unpublished data, 2016). The CF model also recapitulated susceptibility to three of the commonest respiratory pathogens associated with the disease. Gene expression studies showed that potential treatments are effective in the CF model versus the healthy lung model. Most promisingly, ivacaftor, which facilitates increased chloride transport by potentiating open-channel probability in the CF transmembrane conductance regulator, increased this activity in epithelial cells in the CF model.

Microfluidic systems utilising human cells also show promise: a modular microfluidic model of human airways replicated the changes in oxygen tension in different compartments of CF airways [55]. The device was used to study antibiotic treatment of *Pseudomonas aeruginosa*, the bacteria mainly responsible for morbidity and mortality in CF. A mechanically active human lung-on-a-chip microfluidic platform has also been developed that reproduces complex organ-level responses to bacteria (*Escherichia coli*) and to inflammatory cytokines introduced into the alveolar space [56].

3D human tissue constructs can benefit from the use of natural or synthetic scaffolds. Scaffolds create a more life-like microenvironment, supporting cell growth, and encouraging efficient differentiation of hiPSCs. They can also improve cell co-culturing, tissue architecture, and cell functionality [57]. To assess the strengths and weaknesses of 3D models, we need comparisons of those currently available. They include precision-cut human lung slices, used to study replicating human rhinovirus 1b in airway epithelial cells; human bronchospheres, which have different cell layers, beating cilia, and mucus production; human bronchotubules, *in vitro* organoids with lumens that constrict in response to bronchostimulants; and co-culture models of human airways.

These human-biology based models are promising, but to validate their reliability, the data need to be better mapped to human disease subsets, and the 'omics could provide better discrimination for this purpose. Looking to the near future, 3D printed organs might also have an impact. These *in vitro* developments, combined with systems approaches and 'omics analyses, as well as advances in clinical airways imaging, could well revolutionise research into respiratory diseases.

Autoimmune disease

Autoimmune diseases, such as autoimmune vasculitis, are exquisitely human illnesses with complex genetic backgrounds and variable clinical presentation. They range from local to systemic conditions and from acute to lifelong chronic diseases. The underlying pathophysiology is currently insufficiently understood and treatment is mainly empirical, with limited efficacy and significant adverse effects. Until now, autoimmune diseases have been studied mainly using a variety of cell-based *in vitro* assays using relatively simple cultures and in animal models. The cell culture systems lack many elements of clinical disease, and the animal models are intrinsically flawed because the animal immune system, particularly that of rodents, differs in several crucial aspects from the human immune system. Even mouse models based on a 'humanised' immune system lack the complex genetics underlying human autoimmune disease and organ/tissue antigens are nonhuman. This limits recapitulation of the immune response and tolerance mechanisms seen in human disease [58]. Improved understanding of human pathophysiology is essential to develop more effective targeted therapy for personalised treatment, implying a need for human investigational disease models for immune-mediated diseases.

Autoimmune (i.e., antineutrophil cytoplasmic antibody-associated) vasculitis is an example of an immune-mediated disease. Patients with this condition have vascular inflammation with inflammatory leukocytes in blood vessel walls. This results in obstruction of blood vessel lumens leading to downstream ischaemia, tissue necrosis, and bleeding through the damaged blood vessel wall. Microfluidic organ-on-chip systems with hiPSC-derived differentiated cell and tissue cultures open the door to new approaches. They are human specific and allow the independent variation of selected molecular factors and individual cell types, while simultaneously measuring system-level responses in real time [59]. They facilitate long-term co-culture of human cells with properties more closely recapitulating the human *in vivo* situation than did previous, simplistic 2D cell cultures. The essential elements of organ-on-chip models for immune-mediated vasculitis are a dual-chamber chip containing three cell types [human organ-specific (e.g., lung) endothelial cells in culture, immune cells and target organ tissue], a readout system, and a computational disease model that can fit new information gained into broader information on the disease.

In vitro microfluidic chip-based models use advances in human stem cell technology, microfluidics, microelectronics, and microfabrication. The new stem cell technologies enable the derivation of iPSC lines from patients and differentiation to the cell types required. The model can be relatively simple or more complex depending on the research question. Mechanisms of end-organ damage caused by circulating autoantibodies can be investigated with a relatively simple model based on a blood vessel on-a-chip (e.g., [60]) perfused with antineutrophil cytoplasmic antibodies and neutrophils derived from blood. A more complex model incorporating tissues from the immune system is needed to unravel the pathogenesis of autoimmune vasculitis.

Investigation of the innate immune response in immune-mediated vasculitis could be relatively simple because no human leukocyte antigen (HLA) matching between immune cells and target tissue in culture is required. Primary human blood cells (monocytes and neutrophils) can be used or, alternatively, specific cell lines or hiPSC-derived blood cells. Studies of the adaptive immune response need models that include peripheral lymphoid organs, such as the lymph node. Those looking at the development of an immune response or tolerance require culture of primary lymphoid organs, such as the thymus and bone marrow. Alternatively, HLA-identical iPSCs from patients' cells have been used for the culture of the endothelial cell layer, for example. Studying inflammatory responses involves use of tissue- or organ-located dendritic cells and circulating cells, including monocytes and neutrophils. All of these cells and tissues have already been derived from hiPSC *in vitro* (e.g., [61,62]). A human artificial lymph node culture model (HuALN) with relevance to these approaches has been developed as a 3D microfluidic culture system for studying the induction or modulation of cellular and humoral immune responses [63]. HuALN utilises primary human leucocytes from healthy adult donors as the basis of the antigen-dependent, immune-competent lymph node micro-organoids, maintained in a long-term 3D matrix-assisted co-culture system. HuALN has a broad panel of read-out parameters, including metabolics, cellular analytics, antibody secretion, genomics and proteomics, cytokine and chemokine secretion, histology, and imaging.

A human organ-on-chip model for autoimmune vasculitis could be used at various stages in the development process for new drugs and treatments. It would also find applications in the discovery and/or validation of companion diagnostic assays to predict human efficacy and toxicity of lead drug candidates, for the purpose of patient stratification. It would reduce associated costs and should decrease late-stage failures in clinical trials. Investigational work with organ-on-chip and 3D culture systems, combined with knowledge-based computational disease modelling, carries the promise of defining cellular and organ-level disease pathways and filling in missing information on human disease pathophysiology in autoimmune diseases.

So far, the benefits of microfluidic systems have been mainly recognised within a small scientific community. Off-the-shelf availability of microfluidic devices and their components, perhaps on a modular basis with plug-in connections, will make these systems more user friendly. Active dialogue will be needed, particularly with pharmaceutical companies and regulatory authorities, to create wider awareness of the potential of organs-on-chips in research and testing.

Discussion and recommendations

Workshop participants agreed that reviews of therapeutic progress in several disease areas confirmed that continued reliance on animal models is unlikely to improve the currently poor rate of clinical approval of new treatments. There is a pressing need for innovative research roadmaps that focus on understanding human disease pathophysiology and defining disease AOPs at multiple biological levels, from molecular mechanisms to adverse outcomes in patients. They should incorporate 21st-century advances in human-specific tools and models, and funding should be prioritised for the development and validation of next-generation human-based approaches.

An adapted AOP concept, a critical part of the current transition underway in toxicology, would have great value in biomedical research: for defining human pathophysiological pathways for drug discovery and for drug efficacy and safety testing. Disease AOPs would provide clear mechanistic rationales for diagnostic, preventative, and therapeutic interventions, including personalised medicine. This requires strong multidisciplinary collaboration, based on mutual understanding among disciplines such as physics, chemistry, engineering, cell and molecular biology, stem cell biology, clinical medicine, and advanced bioinformatics.

Disease research roadmaps

Disease research roadmaps that focus on human biology-based models and approaches need to be developed, involving a gap analysis to identify what human pathophysiological knowledge is currently lacking in each disease area, and whether we have the technologies and models to acquire it. The AOP concept provides a framework for assembling existing knowledge and identifying those gaps.

In terms of advanced *in vitro* human cell models, several breakthroughs have been made in recent years. hiPSCs allow human-, disease- and patient-specific approaches to studies *in vitro:* the technology is still developing but progress is rapid. Generating high-quality hiPSC lines is time consuming and expensive, although the costs are decreasing, and programming and quality control approaches need to be standardised to improve interlaboratory reproducibility. Microfluidics platforms, as well as 3D culture systems, create reproducible models that recapitulate human disease pathophysiology more completely than before, and are accessible to real-time multiplexed analysis of structure and function. Genome editing *in vitro*

is another recent advance that will be fruitful in identifying gene variations that are causative rather than merely being on the disease pathway of interest. These human-specific *in vitro* models are promising, but require funding for further development and to map the data they produce onto human (clinical) disease information. The reliability of these models could be assessed by using 'omics data. hiPSC-derived culture models will benefit from innovative strategies to collect human biological material and clinical information from large patient cohorts. Biobanks are needed for the large-scale collection, storage, and distribution of human cells and tissues, including normal and patient-derived somatic cells for reprogramming to iPSCs. This will increase the statistical power needed to distinguish information from noise.

The availability of good-quality normal and diseased human tissues, derived postmortem or from biopsies, is essential. Human tissue research can help define key pathological features of human disease against which to validate new *in vitro* models, but there are challenges to obtaining good-quality human tissue, especially from the nervous system. For example, available postmortem brain tissue is often not ideally representative of different stages of disease and, in some countries, patient tissue can only be used to study tightly specified research questions. Several measures can help improve the situation. Local agreements between hospitals and universities or other research institutions would facilitate tissue provision and exchange, and the support of patient advocacy organisations is valuable. In the UK, it is easier to get consent to use human tissue in a more open-ended way, and that experience could be shared with other countries.

Clinical *in vivo* studies with volunteers provide gold-standard data relevant to validating innovative *in vitro* models, especially if valid clinical biomarkers can be found that are applicable to *in vitro* studies.

Computational models have value in integrating multiscale data and linking clinical biomarkers to underlying disease pathways, but more work is needed. A systems approach to researching disease allows the mapping of multiple 'omics data onto tissue samples from patients and their clinical and physiological data. New bioinformatics and mathematical tools help generate novel clinical disease classifications and enable disease-associated pathways to be investigated.

Provision, sharing and organisation of data

A significant contribution to disease research roadmaps would be support for measures such as tools and databases to integrate and share information; encouraging open access publication and data sharing; and the appropriate use of patient data and data from clinical trials. For maximum utility and applicability, it is also essential that data are shared so that all information can be used to improve biological modelling. This can be accomplished by means of a global centralised data or knowledge base. An alternative approach could be the construction of different databases that are seamlessly integrated, for example by use of common templates, ontologies, and integratable platforms.

The handling and curating of big data needs improving. For example, the value of functional genomics would be enhanced if better knowledge management platforms existed and more readily accessible bioinformatics platforms were available for biologists and clinicians to manage and interpret large-scale data sets. Scientists should be encouraged to enter their data into shared, open-source knowledge bases (e.g., the OECD AOP Knowledge Base) to enable vertical and horizontal integration of data within disease areas and across diseases. In the USA, all federally funded research must soon be published in an open format and the underlying data must also be publicly accessible; the EU has given similar stipulations concerning research funded through Horizon 2020.

Kola and Bell published a 'call to reform the taxonomy of human disease' and proposed a new, mechanism-based classification [64]. We identified as a major issue the conceptual and data gaps between *in vitro* findings and clinical knowledge. These gaps need to be bridged to maximise available data and to realise a mechanism- or, better still, pathways-based classification of human diseases. An AOP disease pathway concept provides a means to connect these currently divided fields and provide missing data (Figure 3). Data mining by computer can capture knowledge at all levels of biological complexity, which can help populate disease AOPs. Regulatory agencies should consider offering academic researchers access to their databases and, thus, foster data-mining opportunities. Disease maps and models should be created that build on disease-specific as well as generic information, to enable *in silico* experimentation and the prediction of knowledge gaps. Appropriate anonymisation standards need to be developed. In the EU, there are member state differences over data protection that will take some time to resolve, but it has significant implications for health research. These are important first steps, but more is needed. Funding bodies and policy development could support knowledge bases or platforms where disease pathway-relevant research would be held under a common set of standards, so that data can be submitted in a way that facilitates their use by others.

Policy and funding frameworks

To build on the advances made with human-specific models and to give a clear direction for more effectively incorporating their use into biomedical research over the next decade, strategic policy and funding frameworks are essential. Overarching strategic frameworks would prioritise the funding and deployment of human-specific models, technologies, and infrastructures over the next 5–10 years. Such strategic frameworks would ideally be developed for, and coordinated among, the EU, USA, and other key innovation economies, through a transparent process inclusive of all stakeholders, including academic and pharmaceutical researchers, research-funding bodies, regional and national regulators and advocacy organisations, including health advocacy groups.

Within Europe, the European Commission is well placed to establish high-level strategic bodies to review progress in specific disease areas, identify knowledge gaps and propose adaptations to existing strategies, by providing research funding and coordination activities. Joint Programming Initiatives (http://ec.europa.eu/research/era/joint-programming-initiatives_en.html) already respond to the challenge of coordinating research undertaken at member-state level. They could form the basis of a more centralised approach also involving EU-funded research projects, with the objective being to establish a shared understanding of research needs and opportunities. In the USA, the Tox21 consortium (http://tox21.org/) is an example of a crossdisciplinary approach. Tox21 was established by the EPA, the National Institutes of Health Chemical Genomics Center, the National Toxicology Program, and more recently, the US Food and Drug Administration, in recognition of the failure of animal testing to meet 21st-century regulatory needs. Its strategy includes researching, developing, and validating innovative chemical testing methods that characterise toxicity pathways using new tools. A similar interagency consortium for biomedical research, led by the US National Institutes of Health, would be a valuable development.

It is essential to acknowledge that addressing major societal challenges, such as the current failure rate in drug development, requires new policy approaches in addition to innovative science. Workshop participants recognised concerns expressed by researchers developing novel techniques about the difficulty in obtaining funding for method and model developments that focus solely on human biology, and agreed that conservatism within funding agencies favouring traditional animal models represents a barrier to progress. To stimulate change, the creation and use of human-specific models could be incentivised; the use of animal models and their data should always be scientifically justified. Similarly, it is hoped that any such conservatism among journal editors and reviewers can be overcome as increasing numbers of researchers recognise the relevance of new and emerging non-animal models and technologies.

Concluding remarks

If the goal of biomedical research is to advance human medicine, we must move decisively away from the quest to improve animal models, which are often insufficiently relevant or reliable, and towards the prioritised use of human-biology based methods. A new approach is needed to strategically implement advanced models and tools to understand human disease pathways at multiple biological scales. Funding and policy decisions should drive the development and implementation of 21st-century human-specific scientific approaches, to help ensure that recommendations for disease research roadmaps (Box 1) will benefit every step of the research and drug development process. A better framework for biomedical research, based on mapping human disease pathways at multiple biological scales, would enable new treatments to progress ‘from bench to bedside’ more quickly and cost-effectively.

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Conflicts of interest

G.R.L., E.M., T.S., and C.W. work for Humane Society International, and C.W. also works for Humane Society of the United States. Both organisations have as one of their goals the phasing out of animal use in testing and research. I.M.A. is a principal investigator in the U-BIOPRED consortium, which receives funding from the European Union and from the European Federation of Pharmaceutical Industries and Associations as an IMI JU funded project (no. 115010). The views expressed in this article are those of the authors and do not necessarily reflect the views or policies of their organisations.

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Figure 1. [DE2]Hypothetical 'disease adverse outcome pathway' (AOP). An AOP spans many levels of biological complexity, starting at the molecular level with a molecular initiating event. This is linked causally via key events at cell, tissue, organ, and system levels, resulting in an adverse outcome in the individual. Abbreviation: MIE, AOP molecular initiating event.

Figure 2[DE3]**.** Novel tools and readouts applicable to human-oriented Alzheimer's disease (AD) research at multiple levels of biological complexity. These tools provide information that can be used to map disease pathways. Reproduced, with permission, from [65]. Abbreviations: CSF, cerebrospinal fluid; GEP, gene expression profiling; GWAS, genome-wide association studies; HVLT, Hopkins verbal learning test; IF HCS, immunofluorescence-high content screening; iNCs, induced neuronal cells; iPSCs, induced pluripotent stem cells; MEA, microelectrode array; MMSE, mini-mental state examination; MRI, magnetic resonance imaging; MRS, magnetic resonance spectroscopy; MS, mass spectrometry; NSCs, neural stem cells; PET, positron emission tomography

Figure 3. [DE4]In many disease areas, there is a fundamental disconnect between the concepts, terminology, and data associated with molecular and/or cellular studies and those associated with clinical approaches. The adverse outcome pathway (AOP) framework, applied to biomedical research, would provide the missing links by clarifying key events in disease pathways at all relevant biological scales: molecular, cellular, tissue,

organ, system, and individual. Abbreviation: KE 1, 2, 3, possible key events in an AOP; MIE, AOP molecular initiating event that starts the pathway perturbation.

Box 1. Summary of recommendations

- A 21st-century roadmap for biomedical research and/or a roadmap for each disease area needs to be designed, to provide a strategic approach for utilising human-specific models and innovative technologies.
- One or two research roadmaps could be developed as prototypes, using existing knowledge and making full use of human-specific models.
- We propose a repurposing of the AOP concept in toxicology to form the basis of understanding human disease pathophysiology and improve drug discovery and development in biomedical research.
- Effective strategies are required to collect human biological material and clinical information from large patient cohorts and healthy individuals, to increase understanding of human diseases and assist the validation of new human-specific models *in vitro* and *in silico.*
- Progress with microfluidic systems will require investment to produce off-the-shelf devices and components, perhaps on a modular basis with plug-in connections, to make these systems more use friendly.
- The sharing of ideas, expertise, and concepts between various research communities and clinicians needs ongoing facilitation. 21st-century tools often draw on multiple disciplines and there is frequently a disconnect between research and clinical data, which could be bridged by means of disease pathways.
- Patient groups should also be involved, because they can facilitate science-driven solutions.
- Systems biology approaches, including new bioinformatics and mathematical tools, need further support. They will help integrate multiscale data, generate better clinical disease classifications, enable disease-associated pathways to be investigated, and suggest new research directions.
- New databases are needed to integrate and share information. Open-access publication and data sharing should be not only encouraged, but also required for all publicly funded research. These developments require dedicated funding and policy support. Funding agreements could specify that relevant research findings are to be input into a common global knowledge base, such as the AOP Knowledge Base, and other shared, open-source platforms.
- Effective data mining will be improved by harmonised standards of patient anonymisation and data protection, because this has significant implications for health research.

- Overarching strategic frameworks are essential to direct policy initiatives and funding programs to essential areas that need further development and to coordinate related activities. These frameworks would ideally be coordinated among the EU, USA, and other key innovation economies, through a process of dialogue among all stakeholders.
- To advance 21st-century human-specific scientific progress, funding should be focussed strategically on acquiring critical human information and on developing and validating the necessary new tools, rather than on further developing animal models.
- Publishing and funding biases in favour of animal studies need to be addressed.
- Research-funding bodies are encouraged to prioritise crosscutting efforts to elucidate human disease AOPs using a common framework (e.g., the OECD AOP User Handbook).
- In the EU and USA, the European Commission and NIH, respectively, are well placed to establish high-level strategic bodies to review progress in specific disease areas, identify knowledge gaps, and propose adaptations to existing strategies, by providing research funding and coordination activities.

Box

Figure 1

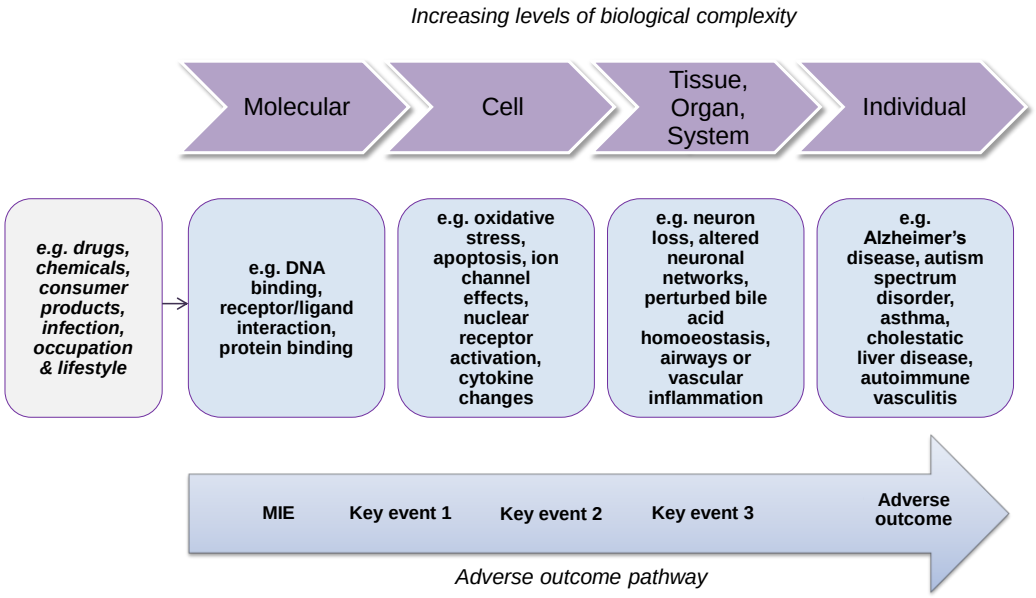

Figure 2

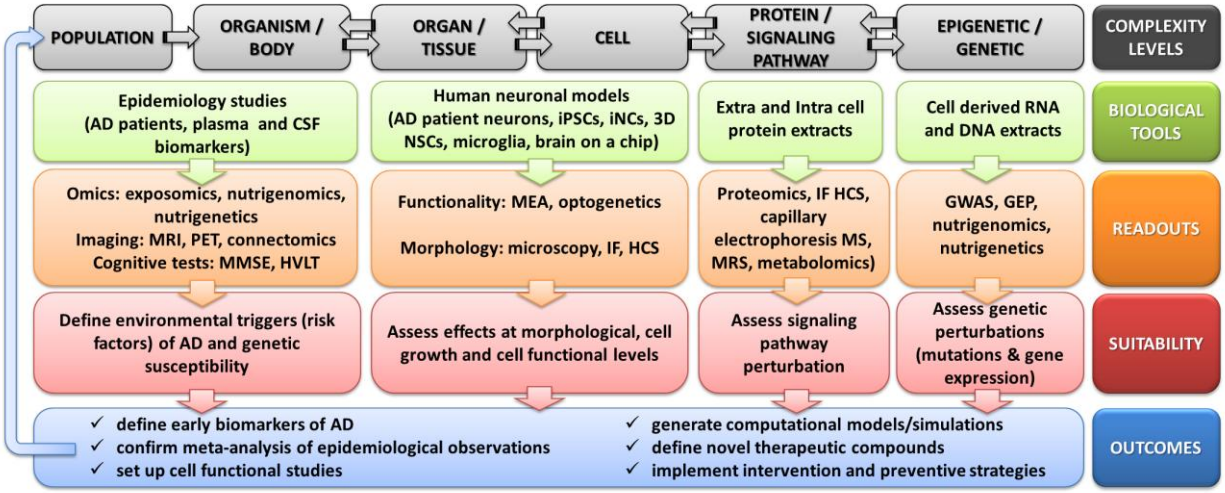

Figure 3